ATTO-TAG™ CBQCA and ATTO-TAG™ FQ

Catalog Numbers A66521, A-6222, A-10192

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Product description

ATTO-TAG™ series of reagents are commonly used for the ultrasensitive detection of primary amines.[1–5] These reagents can be used in applications similar to those of *o*-phthaldialdehyde (OPA), naphthalene-2,3-dicarboxaldehyde (NDA), and anthracene-2,3-dicarboxaldehyde owing to their chemical similarities.[8,9] ATTO-TAG™ CBQCA (3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde or CBQCA[1]) and ATTO-TAG™ FQ (3-(2-furoyl)quinoline-2-carboxaldehyde[2]) also react with hydrophilic peptides and amino sugars to form highly fluorescent conjugates in contrast to *o*-phthaldialdehyde (OPA), and naphthalene-2,3-dicarboxaldehyde (NDA). ATTO-TAG™ CBQCA can be used to determine the amino acid profile of single cells.[9] Detection of amine adducts of ATTO-TAG™ reagents is possible using electrochemical methods[10] or chemiluminescent reagent bis-(trichlorophenyl) oxalate (TCPO).[11-13]

ATTO-TAG™ reagents have the following features:

- **Highly sensitive detection:** Sensitivity of detection is in the attomole range (10^{-18} moles), when using capillary zone electrophoresis (CZE).
- **Minimal background interference:** Ensures that the fluorescence signal obtained is solely from the derivatized analytes.
- **Long-wavelength excitability:** Enables compatibility with various research, diagnostic, and forensic applications, including drug analysis.
- **Versatile compatibility:** Absorbance or fluorescence techniques can be used for detection, providing flexibility in experimental setups. ATTO-TAG™ reagents are also compatible with various chromatography methods, including HPLC.

Contents and storage

The ATTO-TAG™ FQ Plus Amine-Derivatization Kit, supplies sufficient reagents for 200–400 derivatizations. Derivatizations of highly dilute samples ($<10^{-6}$ M) requires an excess of the reagent to complete the reaction.

Table 1 ATTO-TAG™ FQ Plus Amine-Derivatization Kit (Cat. No. A66521)

Item	Amount	Storage[1]
ATTO-TAG™ FQ Derivatization Reagent (Component A)[2]	5 mg	–20°C Protect from light.
Mandelonitrile (Component B)[3]	4 mL	
β-cyclodextrin (Component C)[4]	250 mg	

[1] Products are stable for at least 12 months from date of shipment and stock solutions are stable for at least 6 months after reconstitution when stored as indicated.
[2] The molecular mass of the reagent is 251.2 kDa.
[3] Mandelonitrile is used as an alternative cyano source to potassium cyanide. It is provided as a 20 mM solution in DMSO, which will freeze if stored at or below 4°C. The molecular mass of the reagent is 133.15 kDa.
[4] β-cyclodextrin can be added to electrophoresis buffer to optimize the separation of CBQCA-peptide conjugates. The typical concentration used is 20 mM (23 mg/mL).

Table 2 ATTO-TAG™ derivatization reagents

Item	Cat. No.	Amount	Storage
ATTO-TAG™ CBQCA Derivatization Reagent[1]	A-6222	10 mg	–20°C Protect from light.
ATTO-TAG™ FQ Derivatization Reagent[2]	A-10192	10 mg	

[1] The molecular mass of the reagent is 305.3 kDa.
[2] The molecular mass of the reagent is 251.2 kDa.

Procedural guidelines

- Protocols that use ATTO-TAG™ CBQCA and ATTO-TAG™ FQ are similar. Potassium cyanide (KCN) and mandelonitrile can be used interchangeably at the same concentrations.
- Derivatization reactions are pH dependent (8.5–9.5) and small differences in labeling performance have been observed with the changes in pH and the choice of KCN or mandelonitrile. 0.05–0.01 M Sodium Borate buffer pH 8.5–9.5 is recommended.
- ATTO-TAG™ reagents, commonly employed in chemical derivatizations, must be used at relatively high concentrations to ensure proper reaction kinetics and sufficient modification of the analyte.

Reactions and resulting products

ATTO-TAG™ CBQCA (3-(4-carboxybenzoyl) quinoline-2-carboxaldehyde) and ATTO-TAG™ FQ (3-(2-furoyl) quinoline-2-carboxaldehyde) react specifically with primary amines to form conjugates that are analyzed by electrophoretic or chromatographic methods.

Table 3 Maximal excitation and emission wavelengths of the resulting products

Reagents	Products	Maximal excitation wavelength	Maximal emission wavelength
ATTO-TAG™ CBQCA	Figure 1 - Highly fluorescent 7-aza-1-cyano-5,6-benzisoindoles	450 nm or by the 442 nm spectral line of the HeCd laser	~550 nm
ATTO-TAG™ FQ	Figure 1 - Highly fluorescent 7-aza-1-cyano-5,6-benzisoindoles	480 nm or by the 488 nm line of the argon-ion laser	~590 nm

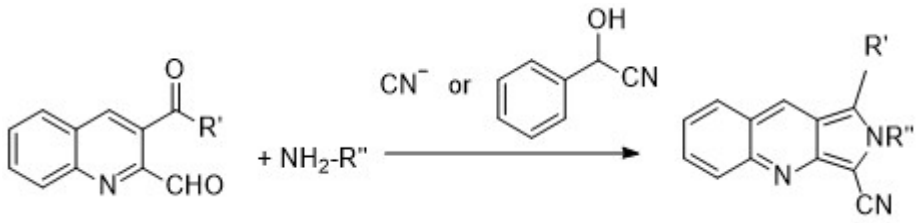

Figure 1 Reaction of the ATTO-TAG™ fluorogenic amine derivatization reagents with primary amines results in highly fluorescent, 7-aza-1-cyano-5,6-benzisoindoles

Prepare working solutions

Prepare ATTO-TAG™ CBQCA and ATTO-TAG™ FQ working solution

Catalog No.	Preparation
(A-6222) ATTO-TAG™ CBQCA derivatization reagent	Prepare a 10 mM ATTO-TAG™ CBQCA working solution by dissolving 10 mg of ATTO-TAG™ CBQCA derivatization reagent in 3.28 mL of Dimethyl sulfoxide (DMSO), then mix thoroughly.
(A-10192) ATTO-TAG™ FQ derivatization reagent	Prepare a 10 mM solution of ATTO-TAG™ FQ working solution by dissolving 10 mg of ATTO-TAG™ FQ derivatization reagent in 4.0 mL of methanol, then mix thoroughly.
(A66521) ATTO-TAG™ FQ Plus Amine-Derivatization kit	Prepare a 10 mM solution of ATTO-TAG™ FQ working solution by dissolving ATTO-TAG™ FQ derivatization reagent in 2.0 mL methanol.

- Thaw the ATTO-TAG™ derivatization reagents at room temperature.
- The prepared working solution must be thoroughly thawed and mixed before each use.
- A working supply of the solution can be stored at room temperature, and it is recommended to replace it on a daily basis to ensure its effectiveness.

Derivatization procedures

The following protocols are based on the publications of Novotny et al.,[1,2,4–7] and constitute general guidelines for the use of the reagent. Most protocols have been developed using CBQCA. Protocols that use ATTO-TAG™ FQ are similar.

Derivatization of amino acids and peptides

For the complete and rapid derivatization of amino acids, using at least sixfold molar excess of ATTO-TAG™ CBQCA or ATTO-TAG™ FQ and a fivefold molar excess of cyanide/mandelonitrile is recommended. For dilute samples, a much larger excess of reagent is required to achieve complete reaction.

1. Mix a 2–5 µL aliquot of the amino acid or peptide at a concentration of 10^{-4} to 10^{-6} M with 5–10 µL of 20 mM KCN/mandelonitrile working solution and 5–10 µL of 10 mM ATTO-TAG™CBQCA or ATTO-TAG™ FQ solution.

2. Allow the mixture to react at 15–30°C for at least one hour.

Note: The optimal pH range for the reaction of amino acids and smaller peptides (up to four amino acid residues) is 8.5–9.5

Stability of the resulting products post derivatization

- The cyanoisoindole derivatives of amino acids and peptides are stable in solution for about 24 hours.
- When evaporated to dryness and frozen, the products are stable for at least two weeks.

Derivatization of amino sugars

The fluorescence intensity of CBQCA and FQ conjugates with amino sugars is appreciably decreased by high concentrations of ATTO-TAG™ CBQCA or cyanide/mandelonitrile. Optimum results are obtained with a one- to twofold molar excess of ATTO-TAG™ CBQCA or ATTO-TAG™ FQ and a one- to threefold molar excess of cyanide/mandelonitrile.

1. Mix an aliquot of the amino sugar with 5–10 µL of the 20 mM KCN/mandelonitrile working solution and 5–10 µL of the 10 mM ATTO-TAG™ CBQCA or ATTO-TAG™ FQ solution.

2. Allow the mixture to react at 15–30 ºC for about one hour prior to analysis.

Note: The ideal pH range for reaction of amino sugars with ATTO-TAG™ CBQCA or ATTO-TAG™ FQ is 8.5–9.3.

Stability of the resulting products post derivatization

- The cyanoisoindole derivatives from amino sugars are stable for at least 10 hours.
- When evaporated to dryness and frozen, the products are stable for at least two weeks.

Derivatization of reducing monosaccharides and oligosaccharides

1. Introduce an excess of 2 M $(NH_4)_2SO_4$ or 4 M NH_4Cl and 0.4 M $NaCNBH_3$ to an aqueous solution of the carbohydrate sample in a screw-cap vial to perform reductive amination procedure

2. After thoroughly mixing the tightly sealed vials, place them in a temperature-controlled heating block and maintain the temperature at 100°C for 100–120 minutes.

3. After completion of the reaction, immediately cool solutions in an ice bath. The mixtures can be used directly for derivatization by CBQCA or FQ as described preciously. If desired, the samples can be dried and redissolved.

Guidelines for separation and detection

Separation and detection of amino acids

- In studies referenced below, conducted in simple ionic buffer systems, roughly half of a group of seventeen CBQCA-derivatized amino acids were successfully separated by capillary zone electrophoresis. By adding sodium dodecyl sulfate (SDS) as a micelle-forming agent, most amino acids are adequately resolved.
- Lysine can be a problem due to double-labeling by CBQCA, leading to fluorescence quenching and spectral changes. In protein hydrolysates, lysine can be analyzed by methylation of the amino side group before hydrolysis. This process methylates the *N*-terminus, allowing detection of lysine by comparing both methylated and non-methylated protein samples after hydrolysis and derivatization.

- Detection limits for various amino acids derivatized with CBQCA range from 20–70 attomoles, with glycine detectable at 1.4 attomoles. Based on the measurements taken, there is a linear dynamic range of over three orders of magnitude.

Table 4 Typical conditions for CZE separation of CBQCA-derivatized amino acids

Item	Conditions[1]
Capillary	50 µm i.d. (184 µm o.d.), 104 cm in length (73 cm to detector)
Mobile phase	0.05 M TES buffer (pH 7.02), 50 mM SDS, 10 seconds hydrodynamic injection, injection concentration 8.7×10^{-6} M
Operating Voltage	25 KV (14 µA)
Excitation	HeCd laser, 50 mw at 442 nm; Emission: 550 nm

[1] These conditions may differ with the uncharged ATTO-TAG™ FQ.

Separation and detection of peptides

- Small peptides and larger angiotensin derivatives are easily separated as their CBQCA derivatives in a borate buffer system at pH 9.5.
- Derivatives migrate based on their expected ratios of mass to charge.
- Lysine-containing peptides, and proteins in general, can exhibit several peaks.
- A small sample of model peptides was tested and the detection limits ranged from 4.6 to 13.8 attomoles. The linear dynamic range was found to be at least four orders of magnitude.
- By incorporating cyclodextrins in the buffer system, CBQCA-peptide conjugates have achieved a significant enhancement of detection sensitivity and narrower peaks.
- The β-cyclodextrin concentration from 0–20 mM causes nearly 10 times increase in the relative fluorescence intensity of larger peptides like angiotensin derivatives, although a tetrapeptide shows a much smaller increase.
- A 10–20 mM of α-cyclodextrin can also enhance the fluorescence of small peptides.

Table 5 Typical conditions for CZE separation of CBQCA-derivatized peptides

Item	Conditions
Capillary	90 cm in length (60 cm to detector), 50 µm i.d.
Mobile phase	0.05 borate buffer (pH 9.5), 20 mM α- or β-cyclodextrin.
Operating Voltage	20 KV

Separation and detection of amino sugars

- In phosphate buffer, the CBQCA conjugates of D(+)-glucosamine and D(+)-galactosamine show identical electrophoretic mobilities. They are readily separated, however, by addition of borate ion to the buffer.
- Complete separation is obtained at borate concentrations of 10 mM.
- Incorporation of anionic surfactants such as SDS as a buffer additive, leads to an improvement in the resolution.

Table 6 Typical conditions for CZE separation of CBQCA-derivatized amino sugars

Item	Conditions
Capillary	80 cm in length (50 cm to detector), 50 µm i.d.
Mobile phase	20 mM Na_2HPO_4/20 mM borate/50 mM SDS, pH 9.12
Applied Voltage	16 KV (28 µA)
Injections	10–15 seconds hydrodynamic

Applications of ATTO-TAG™ reagents

ATTO-TAG™ reagents have additional applications, including:

- Analysis of amine-containing constituents of human cerebrospinal fluid.[14]
- Detection of enantiomeric separations by capillary electrophoresis.[15]
- Detection of localized chemical modifications on molecular monolayers.[16]

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Revision	Date	Description
A	8 May 2024	New document for ATTO-TAG™ reagents